(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) EP 0 705 842 A2

(12) EUROPEAN PATENT APPLICATION

(43) Date of publication:
10.04.1996 Bulletin 1996/15

(51) Int. Cl.[6]: C07K 14/00, C12Q 1/68

(21) Application number: 95115510.0

(22) Date of filing: 02.10.1995

(84) Designated Contracting States:
AT BE CH DE DK ES FR GB GR IE IT LI LU NL PT SE

(30) Priority: 06.10.1994 EP 94115751

(71) Applicant: HOECHST AKTIENGESELLSCHAFT
D-65929 Frankfurt am Main (DE)

(72) Inventors:
- Bartnik, Eckart, Dr.
D-65205 Wiesbaden (DE)
- Margerie, Daniel, Dipl Biol.
D-60320 Frankfurt (DE)

(54) Regulated genes by stimulation of chondrocytes with 1L-1beta

(57) The present invention refers to the novel use of osteopontin, calnexin and TSG-6 gene product in the diagnosis, prophylaxis or therapy of IL-1β mediated diseases of connective tissues and to novel genes induced or repressed by stimulation of chondrocytes with IL-1β and their use in the diagnosis, prophylaxis or therapy of IL-1β mediated diseases of connective tissues.

EP 0 705 842 A2

Printed by Rank Xerox (UK) Business Services
2.10.4/3.4

and the DNA TTU2/2 with the sequence

```
AACCAGTATT TCAAAACTAT TATCTGGATT CAAGATTAGT GTGTAAAGAT TGTTTTCTTA   60
TCAGTAAAAT AGGTCTTCAG ATCTGCATCT GGCCTCTTAG CATGTTTTTC TTCATAGATA  120
CCCGTTTTGG GGTTTTTGCG TCGGAAGATG AATGGCATTT ATAGTCCTCT CCACATTTAT  180
CTG                                                                183
```

are 100 % identical to human osteopontin cDNA and 97.2 identical to human calnexin, respectively. This demonstrates that the experimental approach of the present invention worked efficiently, i.e. the use of 100 different primer combinations (25 oligodecamer primers, $4T_{12}$MN-primers) generated a total of approximately 10.000 PCR products for each population which represent 53 % of all expressed cellular genes. 123 PCR bands out of 10.000 appeared as differentially expressed bands. 53 of the original 123 PCR bands were reproducibly displayed by comparing the PCR band patterns from two patients; of those 68 % arose from IL-1β stimulated chondrocytes.

It was further found that osteopontin which is a secreted highly acidic phosphoprotein of 32 kd (Denhardt and Guo (1993) FASEB J. 7, 1475-1482) is surprisingly downregulated in IL-1β stimulated human chondrocytes. This means that osteopontin is involved in IL-1β related diseases of connective tissues, in particular osteoarthritis.

Osteoarthritis is characterized as a slowly progressing matrix degeneration with continuing degradation of collagens and proteoglycans and subsequent release of matrix fragments into the synovial fluid. Any disturbance of the normal chondrocyte matrix interactions, for example through a loss of osteopontin, could cause an altered signaling through the integrin alpha$_v$beta$_1$ and thus changed cellular responses leading to early steps of matrix degradation.

Therefore, one embodiment of the present invention is the use of osteopontin itself or parts thereof, antibodies against it or nucleic acids such as DNA or RNA or parts thereof coding for osteopontin or parts thereof in the diagnoses, prophylaxis or therapy of IL-1β related diseases of connective tissues, in particular osteoarthritis. According to the present application the term "parts" means either at least 8, preferably 12, in particular 15 amino acids in case of proteins or 6-100, preferably 10-40, in particular 12-25 nucleic acids in case of DNA or RNA as hybridization probes. The methods of diagnosing such diseases will be described infra. In addition, quantification on the protein level is possible with osteopontin specific antibodies on Western blots, in immunochemistry, FACS analysis or ELISA based assay systems. The present invention refers also to a diagnosis aid or a pharmaceutical for such use. Osteopontin can be produced for example recombinantly through expression in procayotes, in insect cells in mammalian cells or in mammalian cells using Vaccinia as detailed in Ausubel et al. 1994 [Current protocols in molecular biology, Chapter 16, John Wiley & Sons, Inc]. The cDNA of Osteopontin is e.g. disclosed in Young et al. (1990), Genomics 7, 491 - 502.

Antibodies against osteopontin can be generally produced for example by the method of Neil GA & Urnovitz HB (Trends in Biotechnology, 6, 209-213, 1988) or Köhler G & Milstein C (Nature, 256, 52-53, 1975).

Also calnexin which is an integral membrane protein of 88 kd (Bergeron et al. (1994) TIBS 19, 124-128) is surprisingly downregulated in IL-1β stimulated human chondrocytes compared to unstimulated chondrocytes. This means also that calnexin is involved in IL-1β related diseases of connective tissues, in particular osteoarthritis. In addition, a downregulation of the calnexin synthesis would cause a reduced amount of correctly and completely folded proteoglycans because calnexin is a new type of molecular chaperone that associates with incompletely folded proteins such as proteoglycans. Proteoglycans are highly glycosylated glycoproteins which are of central importance for the maintenance of the cartilage tissue integrity.

Hence, an additional embodiment of the present invention is the use of calnexin itself, or parts thereof antibodies against it or nucleic acids such as DNA or RNA or fragments thereof coding for calnexin or parts thereof in the diagnosis, prophylaxis or therapy of IL-1β related diseases of connective tissues, in particular osteoarthritis. The methods of diagnosing such diseases are already described above. The present invention refers also to a diagnosis aid or a pharmaceutical for such use.

Calnexin can be produced for example recombinantly as described above for osteopontin. The cDNA of Calnexin is e.g. disclosed in Galvin et al. (1992), Proc. Natl. Acad. Sci. USA 89, 8452 - 8456. The production of said antibodies are also generally described above.

Potential role of identified cDNA fragments in IL-1 mediated cellular processes TSG-6

A homology search in the GenBank and EMBL databases revealed a 99.5 % sequence indentity of fragment TAU7/2(c) with the gene coding for human TSG-6. TSG-6 (TNF stimulated gene 6) was originally isolated by differential cDNA library screening as a TNF induced gene sequence from human fibroblasts (Lee et al., 1990). It was further characterized by Lee et al (1992) as a TNF and IL-1 inducible, secretory, 39 kDa glycoprotein with extensive sequence homology with a region implicated in hyaluronate binding, present in cartilage link protein, proteoglycan core proteins,

Therefore, another embodiment of the present invention is a DNA containing a DNA selected from the group consisting of

TA08/2(2)

```
   1   CCAAGTTTTT  CCAGCAACCC  CAAGGGAATA  CAGGGAGATC  AATGCACCA
  51   AAATGGGAAA  AGAAAAATAC  TTCGATGCAA  TGAAACAAAG  CCTTTTTCCG
 101   TTCAGTTTCC  ATAATTCAGT  GGTCAGTTTT  AAGGCTGCCA  CTTGGG
```

TA016/1(2)

```
   1   GACACGAACA  CCACATATTT  TTATTGGAGG  CCCCATGGCT  CCTTGGAAGC
  51   CATTTTGGAA  CCAAGGGGAC  CCACCTTTTT
```

TA016/2(2)

```
   1   CTAAATATAT  TCTCTAACAA  GTTAATCTCT  TTCAAATCTA  TAGATAAAAC
  51   TAAAAGGATA  AGGAACCAAG  GTTTAACCGA  CCTAGCCAAT  TATGGCAATC
 101   ATACTTGCTT  TTTAG
```

TAU 7/2(C)

```
   1   CCTTGAAGAT  GACCCAGGTT  NCTTGGCTGA  TTATGTTGAA  ATATAGACA
  51   GTTACGATGA  TGTCCATGGC  TTTGTGGGAA  GATACTGTGG  AGATGAGCTT
 101   CCAGATGACA  TCATCAGTAC  AGGAAATGTC  ATGACCTTGA  AGTTTCTAAG
 151   TGATGCTTCA  GTGACAGCTG  GAGGTTTCCA  AATCAAATAT  GTTGCAATGG
 201   AT
```

TAU10(1)

```
   1   GGAGATGACA  TTTGCTTTGG  GCAGAGGCAG  CTAGCCAGGA  CACATTTCCA
  51   CTATAATTTT  ACAAAGTTAA  ATTTATAAGC  TAGCATTAAG  TAAAGTGAAG
 101   TTCCAGCTCC  CTTGCTAAAA  ATAACTAGAG  GTAATAATTG  GTATTCAGGT
 151   AACTCATTTA  CATCATAATG  TGTTGTGAAA  A
```

TAU12/1(2)

```
   1   TATAAAATAT  AAATTATATT  ATAAATCATG  TATTATTTAT  AAAATTATAT
  51   TATAAATTTA  TAAAAATATA  AATTATATTT  TAGGCTTAAT  GTATAAGGAA
 101   TATAAATTAT  TAATAAGCAT  ATGA
```

TAU 12/1(1)

```
   1   TGTAATTAAC  TGTNCTTGTA  GGTGTGTCTT  TTATACATGT  GTGAGTTTTT
  51   CTTTACAATA  GATTCCTAGC  ATTGGGATTG  CTAGGTCAGA  TGGTATGCAC
 101   ATTTGACATT  TTGATTGATA  GCACCAGATT  GCTTTGTTAA  AAAATTTTNN
 151   TTTATAGTTT  ACATTATCTT  TGTACAATAG  ATGTTCTCTT  TCGAC
```

TAU 12/2(1)

```
   1   GGGAAGTGAA  TTGAAAATAC  TTCTTTNTCA  ACATAATTTT  NGGGTTTTGA
  51   AATTGTGTTT  GGGTTTTCAG  GAAATTGGTG  GTAATCTTGT  ATTAGACTGAA
 101   AAAAAGTGAA  TTTTAAAATT  CTCAGTGAAG  AAGCAAATGA  TTTATTTTTC
 151   ATAGA
```

TAU12/3(2)

```
   1   TGTTCTGGTA  ACTGTTCTAA  TTGTGTCTTT  GTTACTTCCA  GTGCAACCCT
  51   TTCAGGTAAG
```

TAU12/3(1)

```
   1   CTAAAGAACT  TGGTATCTCT  ATTAAAGCAC  ACGAACCTCC  AAGGAAAATA
  51   GAGCGATTTA  CTCTTCTCAT  ATCAGTGCAT  ATTTATAAGA  AGCACGGAGT
 101   CA
```

TAU13/1(1)

```
   1   AGTCATCAAT  TCCTTTTTAT  CTGTAATTAC  ACATTTGTTT  TTATTTCAAA
  51   GTAATTATAA  GGTGTTATAT  TGCATATAAT  CAGAAAACTA  AATGGAAATA
 101   AAATTTTAGT  AAGCCCGGCC  CCTTTGACCG  ATACAGAAAA  CTTGA
```

TCU2/2(1)

1	CGGGTTAATA	TTATCCTCTA	GTATAAGTGA	ATTACTAGTT	TCTCTTTATT
51	TAGACAAACA	CACACACACC	AGATAATATA	AACTTAATAA	ATTATCTGTT
101	AATGTAGATT	TTATTTAAAA	AACTATATTT	GAACATTGGT	CTTTCTTGGA
151	C				

TCU9/1(2)

1	ACATAACAGC	TTTTATACAA	TGATAAGGAC	ATATCATTTG	TTTACAAAGA
51	AAGTCTAAAA	TTTCAAGAAC	ATTCAAAGAG	CTAACACAGT	AAAGGTCATG
101	CAAGTTCTAG	AATAGTGAAT	CATGACAGAA	CTCATTCATT	TTATCCTTTA
151	TCTCC				

TCU9/2(2)

1	AAGTATGGGT	AGCTAAATTT	GCATTAAATT	AAAAGTACAT	ATAATGCAAC
51	ACCACTCTAC	ATCTGTATAC	CTACGAATGT	ATGTGTACTA	CACACCCTTA
101	AAATGTTTTT	CAAAGTCTTA	ATATATTAGA	ACATGTTTTC	ATTTTTTCAT
151	GGGATGTTAA	TACTATTCTA	TGATTAAGAA	AATACTAG	

TCU10(2)

1	AATACAGTTA	TTCTAGCTTT	TCATATTCAA	TTTGAATGAT	CAGAAAAGTA
51	TATTAGTCAC	ACAGAATTAA	ATATTTTAGA	TAGTAAGAAT	C

TCU14(2)

1	GAAGTGAAAG	TCAGCCCTTT	AGCTATTATT	TATTGCTTTA	TTAGAGCAGA
51	GGGAAGTGAC	ACTCATTGCC	TTCACAGAGC	TCTGCAGAAA	TATATGCACA
101	GAGTGGTCAA	TGCCAACATC	TGAGTAAGTC	TTCCAAA	

TGO20(2)

1	CAGAACATTA	GGATTTATTC	CTTGATTAGT	TCAAATGATT	TCAACAGCTG
51	AATTCCTTGA	GATGTGTAAG	GCAGGTTGGT	CCTTTGGATG	GACTGTAGAC
101	TGAAACTTCC	TATAACTGTA	GTGATATGTA	CACAGCTACA	TAGCAAAGTG
151	CTTCATTATG	AAAATGAAGA	A		

TGO20(1)

1	CAGTGTGAGA	GTCTCATTTC	TATGCACAGT	GTTTCTCAGG	AGGATGGAGC
51	TAGTTAGCTG	TCTGTTGTCT	GTAGCCCAGC	TTGATAATGG	AACTATACAG
101	CGAAGAGACA	ATCTCTGGCA	AGTTTTTGTA	GAA	

TGU5(C)

1	TTAGAGTAAA	ATTCCAAATA	AATGCTTTGC	TCCAAAATTA	CACTAACCAG
51	GCTGGGTCTC	TATCATACAT	CTTCAATACC	CTCAAACCTA	GATTGTAAAG
101	TGAAAAAAGT	GATTAGCNNT	TCCATTTGTT	CATTCTGTCA	CTCACATTCT
151	TAGGCATTTT	AAGGATGAGC	AACCTTTGTT	TCAGAAAGGG	TAAGTAATTA
201	GCCCCCTGGA	GGTTACATAG	TTATAATTTA	GTCTTCAGAA	TCCGTTCGAA

```
      201   CTNAAATTCA  AACACCATGG  CAANAGAAAC  TGCTTCTAT

TTO20/1(C)
        1   CCACCAGCCT  ACTGATCAGC  TGGGATGCTC  CTGCTGTCAC  AGTGAGATAT
       51   TACAGGATCA  CTTACGGAGA  AACAGGAGGA  AATAGCCCTG  TCCAGGAGTT
      101   CACTGTGCCT  GGGAGCAAGT  CTACAGCTAC  CATCAGCGGC  CTTAAACCTG
      151   GAGTTGATTA  TACCATCACT  GTGTATGCTG  TCACTGGCCG  TGGAGACAGC

      201   CCCGCAAGCA  GCAAGCCAAT  TTCCATTAAT  TACCGAACAG  AAATTGACAA
      251   ACCATCCCAG  ATGCAAGTGA  CCGATGTTCA  AGACAACTGT  TTTAATAAAA
      301   GATTTACATT  CCAC

TTO20/2(2)
        1   TTGGTACCAC  AGTCACAGAA  CTGGGGGTCA  TTTTCTAGAT  GAAACAAACG
       51   GAACAAGTTC  TCTTCCAACA  AAGAAATGTA  CTGTAGAAAT  TAATTTCCTC
      101   CATGAATTTT  ATATATTGTG  TACAAATATA  AGGTATGTAT  CTGAATACAA
      151   AG

TTU2/1(2)
        1   CTAGAACTTC  CAAAGGCTGC  TTGTCATAGA  AGCCATTGCA  TCTATAAAGC
       51   AACGGCTCCT  GTTAAATGGT  ATCTCCTTTC  TGAGGCTCCT  ACTAAAAGTC
      101   ATTTGTTACC  TAAACCTTAT  GTGCCTTAAC  AGGCCAATGC  TTCTCG

TTU 2/2(C)
        1   AACCAGTATT  TCAAAACTAT  TATCTGGATT  CAAGATTAGT  GTGTAAAGAT
       51   TGTTTTCTTA  TCAGTAAAAT  AGGTCTTCAG  ATCTGCATCT  GGCCTCTTAG
      101   CATGTTTTTC  TTCATAGATA  CCCGTTTTGG  GGTTTTTGCG  TCGGAAGATG
      151   AAGTGCAGTT  TATAGTCCTC  TCCACATTTA  TCTG

TTU3(1)
        1   GGGTAGAAAG  CTGAATAATT  TATGAAGGAG  AGGGGTCAGG  GTTGATTCGG
       51   GAGGACCTAT  TGGTGCGGGG  GCTTTGTATG  ATTATGGGCG  TTGATTAGTA
      101   GTAGTTACTG  GTTGAACATT  GTTTGTTGGT  GTATATATTG  TAATTGAGAT
      151   TGCTCGGGGG  AATAGGTTAT  GTGATTAGGA  GTAGGGTTAG  GATGAGTGGG
      201   AAG

TTU 5/1(2)
        1   GACAAAAAAA  AAAAAACAGG  TTTTAAAGCT  AGAAATGAAA  AGCTACTTAA
       51   GTATCTTAAA  GGATAAGTTA  CTTTATTATA  CACTAGAAAC  ATACACAATA
      101   GCTGAAAACT  TAAAAAATCT  CACACTGCTG  AATGTCTCTG  CTGGCTG

TTU5/2(2)
        1   GCATCCATTG  TACATTGTTT  GGTTTGAGGT  TACCATGAGG  CCTGTAAATA
       51   CTATCTTATA  ATTTATTATT  TCAACCTGAT  AAAACTTAAC  ACTATTTGCA
      101   TAAACAAACA  AACGAAAA
```

(b) expressing said gene in a suitable host cell such as BL21 series (Studier et al., 1990, supra) for procaryotic expression or COS, cells for mammalian expression (Aruffo and Seed, 1987, supra) or any other expression system known to one skilled in the art;

or a method for producing a protein containing the steps:

(a) culturing a suitable host cell, in particular the above mentioned, containing a vector, in particular an expression vector such as the vectors mentioned above which contains a DNA or a gene of the present invention; and

(b) isolating the expressed protein for example by ultrafiltration, precipitation with chaotropic agents such as urea or column chromatography on e.g. ion exchange chromatography columns as detailed in Ausubel et al. 1994 (supra).

A further embodiment is a diagnostic aid containing a DNA or parts thereof or a gene or parts thereof of the present invention. In particular, quantification of the genes can be achieved on the RNA level by Northern blotting with gene specific probes of the present invention or with gene specific primers in a PCR reaction. Such primers can be synthetically produced using the DNA sequences of the present invention or the sequences of the corresponding genes. Therefore, said nucleic acids are useful for the diagnosis of IL-1β related diseases of connective tissues, in particular osteoarthritis or rheumatoid arthritis.

These nucleic acids can also be used to evaluate the expression of certain genes in small cartilage biopsies and to use these ultimately as disease-specific markers and/or as predictive markers for disease progression of e.g. osteoarthritis. The hybridization conditions can be the same as described above.

Said nucleic acids, however, can also be used for the therapy against the diseases mentioned or for the production of a pharmaceutical.

Therefore, another embodiment of the present invention is also the use of said nucleic acids for the production of a pharmaceutical. For example, as described by Uhlmann & Peyman (Chem. Rev. (1990), 90, 543), Milligan et al. (J. Med. Chem. (1993), 36, 1923) or Stein & Cheng (Science (1993), 261, 1004) such nucleic acids can be used as antisense oligonucleotides or triple helix forming oligonucleotides for the inhibition of gene expression. This is in particular useful if such a disease is caused by the overproduction of a gene product which is directly or indirectly regulated by IL-1β in chondrocytes. The nucleic acids can additionally be modified in order to increase e.g. the stability against nucleases as described e.g. in the literatures mentioned above.

Finally, also the gene product itself produced by a method of the present invention can be used as a pharmaceutical.

In the following the invention is in particular described by the examples and tables:

Description of the Tables

Table 1 gives an overview on used primers and the complexity of the detected differences in expression.

Table 2 summarizes the result of the sequencing of differentially displayed PCR products after their elution from the sequencing gel, reamplification and subcloning into the pCRII vector. The sequences of TAU1/1(1) and TAU1/1(2) are 100 % identical to human osteopontin cDNA, the sequence of TTU2/2 is 97.2 % identical to human calnexin. bp = base pairs, IL-1 = Interleukin-1 stimulation, Stat. sig. score = statistical significance score: a feature of the BLAST database searching program. This score is determined using an implementation of Karlin's significance formula (Karlin, S. and Altschul, S.F. 1990. Methods for assessing the statistical significance of molecular sequence features by using general scoring schemes. Proc. Natl. Acad. Sci. USA, 87:2264-2268), which calculates the Poisson probability that the observed sequence similarity will occur by chance based on the size and composition of the sequence database as well as on the size and quality of the match. The smaller this number, the more it is likely to see sequence similarities.

Examples

Cell culture

Articular cartilage specimen were obtained from two patients (male 65 years old and female 73 years old) undergoing total joint replacement surgery for osteoarthritis. None of these individuals had received treatment by radiation or chemotherapy. Articular cartilage slices were aseptically dissected from both femoral condyles, tibia plateaus and pattellae and subjected to sequential enzymatic digestion with pronase and collagenase as described (Häuselmann HJ et al. 1992, Matrix 12, 116-129) Since it is known that the alginate gel suspension system retains the chondrogenic phenotype [Lohmander LS et al. 1992, Trans. Orthop. Res. Soc. 17, 273.] 4 x 10^6 chondrocytes were suspended in low viscosity alginate (4 x 10^6 cells / ml 1,25 % w/v alginate in an isotonic buffered solution) and expressed through a 22gauche needle into 102 mM CaCl solution to form cell entrapping beads which are 1,5-3 mm in diameter and spherical. Alginate beads containing a total number of 2 x 10^7 cells were fed daily for the first three days with medium F12 / DMEM (50/50)

List of all degenerate 3' oligo dT-primers [T_{12}VN] used for DDRT-PCR:

Primer	Sequence 5' to 3'
T_{12}VA	5'-TTTTTTTTTTTTVA-3'
T_{12}VA	5'-TTTTTTTTTTTTVT-3'
T_{12}VA	5'-TATTTTTTTTTTVG-3'
T_{12}VA	5'-TTTTTTTTTTTTVC-3'
V = dA, dG, dC; N = dA, dT, dG, dC	

List of all arbitrary 5' oligodecamer primers used for DDRT-PCR:

Primer	Sequence 5' to 3'
OPA 6	GGTCCCTGAC
OPA 7	GAAACGGGTG
OPA 8	GTGACGGGTG
OPA 9	GCGTAACGCC
OPA 10	GTGATCGCAG
OPA 16	AGCCAGCGAA
OPA 17	GACCGCTTGT
OPA 18	AGGTGACCGT
OPA 19	CAAACGTCGG
OPA 20	GTTGCGATCC
U1	TACAACGAGG
U2	TGGATTGGTC
U3	CTTTCTACCC
U4	TTTTGGCTCC
U5	GGAACCAATC
U6	AAACTCCGTC
U7	TCGATACAGG
U8	TGGTAAAGGG
U9	TCGGTCATAG
U10	GGTACTAAGG
U11	TACCTAAGCG
U12	CTGCTTGATG
U13	GTTTTCGCAG
U14	GATCAAGTCC
U15	GATCCAGTAC

Northern-blot analysis

Cell culture and isolation of RNA was performed exactly as described above. 10 µg of total RNA from both IL-1β stimulated or not stimulated chondrocytes were denatured by heating at 65°C for 10 min in a solution of 50 % formamide, 20 mM MOPS and 2.2 M formaldehyde, separated through a 1 % agarose gel containing 2.2 M formaldehyde in 1 X MOPS and transfered to positively charged nylon membrane (Amersham) by standard blotting procedures [Maniatis et. al 1992]. After UV crosslinking, the blots were prehybridized for 1 h in rapid-hyb-buffer (Amersham) at 65°C. A 330 bp cDNA corresponding to nts 61 to 390 of human osteopontin cDNA (GenBank J04765) and a 340 bp cDNA corresponding to nts 881 to 1220 from human calnexin (GenBank M94859) were radiolabeled for hybridization with α-[^{32}P]dCTP (3000 Ci/mmol, 10 mCi/ ml) using random nonamer primers (Amersham) up to a specific activity of ~ 1,5 x 10^9 dpm / µg DNA. Hybridization was performed for 2,5 h at 65°C in prehybridization solution with 2 ng / ml of labeled probe added. The blot was subsequently washed in 2 X SSC, 0.1 % SDS at 37°C for 15 min (1 X SSC = 0,15 M NaCl, 0.015 M sodium citrate, pH 7,0), followed by two sucessive washes with 1 X SSC , 0.1 % SDS at 65°C for 10 min respectively. If necessary, a final high stringency wash was performed with 0.1 X SSC , 0.1 % SDS at 65°C for 15 min. The blots were then analysed by autoradiography using Kodak X-Omat films at -80°C with intensifying screeens for 2-7 days and intensity of bands was quantified with a phosphorimager (Biorad, model GS-250). All blots were stripped with boiling 0.5 % SDS solution and reprobed with labeled β-actin to demonstrate equal loading of RNA in each lane.

Northern hybridisations (Results)

Fragment TAU7/2(c), identical to TSG-6, was differentially upregulated in IL-1 stimulated cells. This is in concordance with Lee et al. (1992) which reported for TSG-6 a TNF-α and IL-1 mediated upregulation. Fragment TAU1/1, identical to human osteopontin and fragment TTU2/2, identical to human calnexin, both were weaker expressed in IL-1 stimulated chondrocytes compared with the unstimulated cells. To validate our differential display data, we performed Northern analyses of Osteopontin and calnexin expression in IL-1 stimulated and unstimulated chondrocytes originating from a third patient. Both messages were again downregulated. A phosphorimager quantification revealed an osteopontin downregulation by 79% and a calnexin downregulation by 40% in the RNA population from chondrocytes of the third

Table 2 IL-1 mediated differentially displayed cDNA fragments of human articular chondrocytes

Fragment	bp	IL-1	Features	Stat.sig.score
TAO 8/2(2)	275 bp	+	146 bp sequenced, no homology found	0.999
TAO 16/1(2)	450 bp	+	80 bp sequenced, no homology found	0.69
TAO 16/2(2)	200 bp	+	115 bp sequenced, no homology found	0.04
TAO 17(c)	412 bp	+	412 bp sequenced, no homology found	0.016
TAO 19(c)	209 bp	--	209 bp sequenced, no homology found	0.99
TAU 1/1(1,2)	450 bp	--	100 % sequence identity to human osteopontin cDNA in 303 bp overlap (303 bp seq.)	1.2 x 10-101
TAU 1/2(2)	430 bp	+	188 bp sequenced, no homology found	0.82
TAU 7/1(1,2)	500 bp	+	87 % sequence identity to human cDNA clone c-1sd02 in 125 bp overlap (235 bp seq.)	8,1 x 10-33
TAU7/2(c)	202 bp	+	99.5 % sequence id to human TNF stimulated gene-6 in 202 bp overlap	4.8 x 10-76
TAU 10(1)	400 bp	+	181 bp sequenced, no homology found	0,9997
TAU 12/1(1,2)	470 bp	--	319 bp sequenced, no homology found	3.3×10^{-14}
TAU 12/2(1)	390 bp	--	155 bp sequenced, no homology found	0.0078
TAU 12/3(1,2)	250 bp	--	95 % sequence identity to human cDNA clone HRBBA21 similar to S10 in 158 bp overlap (162 bp seq.)	1.0×10^{-28}
TAU 13/1(1)	600 bp	+	145 bp sequenced , no homology found	0.12
TAU 13/3(1,2)	500 bp	--	439 bp sequenced, no homology found	0.33
TCO 16/1(c)	241 bp	+	241 bp sequenced, no homology found	2.4×10^{-7}
TCO 16/2(c)	230 bp	+	230 bp sequenced, no homology found	4.3×10^{-5}
TCO 17(c)	169 bp	+	169 bp sequenced, no homology found	0.49
TCO 18(c)	168 bp	+	168 bp sequenced, no homology found	1.3×10^{-6}
TCU 2/1(1)	400 bp	+	178 bp sequenced, no homology found	0,66
TCU 2/2(1)	210 bp	+	151 bp sequenced, no homology found	0.0074
TCU 9/1(2)	430 bp	+	99 % sequence identity to human cDNA clone 131036 3' in 155 bp overlap (155 bp seq.)	$7,2 \times 10^{-58}$
TCU 9/2(2)	320 bp	--	188 bp sequenced, no homology found	0,22
TCU 10(2)	320 bp	--	100 % sequence identity to human cDNA clone 26518 3' in 85 bp overlap (91 bp seq.)	$2,9 \times 10^{-28}$

Fragment	bp	IL-1	Features	Stat.sig.score
TTU 9/1(1)	350 bp	+	94 % sequence identity to human cDNA clone 83764 3' in 159 bp overlap (159 bp seq.)	$5,9 \times 10^{-23}$
TTU 9/2(2)	320 bp	–	149 bp sequenced, no homology found	0,22
TTU 13(1,2)	350 bp	+	194 bp sequenced, no homology found	0,57

Thus, the 44 identified fragments can be subdivided as follows:

1) 2 fragments with sequence homologies to known human genes with known roles in IL-1 mediated processes:

TAU 7/2 identical with human TNF-stimulated gene-6
TTO 20/1 identical with human fibronectin

2) 6 fragments with sequence homologies to known human genes, whose function in IL-1 mediated processes can be speculated:

TAU 1/1 identical with human osteopontin
TGU 8 identical with human 28S ribosomal RNA gene
TGU 13/2 identical with human F1 ATPase β-subunit
TTO 16/2 identical with human ERCC5
TTU 2/2 identical with human calnexin
TTU 3 identical with human NADH-DH mtDNA subunit

3) 9 fragments with sequence homologies to human genes, identified in human geneome sequencing projects:

TAU 7/1 identical with human cDNA clone c-1sd02
TAU 12/3 identical with human cDNA clone HRBBA21
TCU 9/1 identical with human cDNA clone 131036 3'
TCU 10 identical with human cDNA clone 26518 3'
TCU 14 identical with human cDNA clone HL60 3' directed MboI
TGU 9/2 identical with human cDNA clone 12A10B
TGU 12 identical with human cDNA clone 113442 3'
TTU 2/1 identical with human cDNA clone 118470 5'
TTU 9/1 identical with human cDNA clone 83764 3'

4) 27 fragments without sequence homologies to known human genes The detection of TSG-6 and fibronectin, both genes known to be upregulated by IL-1, points to the importance of those other cDNA fragments in the light of IL-1 mediated processes. Those genes very likely play roles in degenerate joint diseases, including rheumatoid and osteoarthritis and with this are interesting candidates as markers for clinical studies or as drug targets for pharmacological intervention.

Claims

1. Use of osteopontin itself or parts thereof, or antibodies against osteopontin or parts thereof or nucleic acids or parts thereof coding for osteopontin or parts thereof in the diagnosis, prophylaxis or therapy of IL-1β a mediated diseases of connective tissues, in particular osteoarthritis.

2. Diagnostic aid for the diagnosis of IL-1β mediated diseases of connective tissues, in particular osteoarthritis, containing osteopontin itself or parts thereof, or antibodies against osteopontin or parts thereof or nucleic acids or parts thereof coding for osteopontin or parts thereof.

10. DNA containing a DNA selected from the group consisting of

```
TAO8/2(2)
       1  CCAAGTTTTT CCAGCAACCC CAAGGGAATA CAGGGAGATC AATGCACCCA
      51  AAATGGGAAA AGAAAAATAC TTCGATGCAA TGAAACAAAG CCTTTTTCCG
     101  TTCAGTTTCC ATAATTCAGT GGTCAGTTTT AAGGCTGCCA CTTGGG

TAO16/1(2)
       1  GACACGAACA CCACATATTT TTATTGGAGG CCCCATGGCT CCTTGGAAGC
      51  CATTTTGGAA CCAAGGGGAC CCACCTTTTT

TAO16/2(2)
       1  CTAAATATAT TCTCTAACAA GTTAATCTCT TTCAAATCTA TAGATAAAAC
      51  TAAAAGGATA AGGAACCAAG GTTTAACCGA CCTAGCCAAT TATGGCAATC
     101  ATACTTGCTT TTTAG

TAO17(C)
       1  CATGAAATAT TTCTTGAGGT AATAAGCTTT TACCAAGCTT ATATTTTTGG
      51  GCAATTCAGT TACAATGAGA AAAAAACACA CCAAAAGACC AAAAATTTTA
     101  AAAACTCACT TTTCTTGCAA TCATAGACAT TTGCATTATT ATAGAACATT
     151  CAAACAAGTT AGGTGGATAA TTATTGTCTA TAGATAAATA CGATGCAATT
     201  TTAATAAGAA TTTGAAGAAT GACATTAAAT GCTGTCTGAA GCCTTTGTAT
     251  TTTTTAATGT ATGACCGATA CTCCGTATAT ACTTAGATAA CTTATCCAGA
     301  AACCTCAACT GTATTGAACA TTGCTGAGAG AAATCAACAA TAATTTTAAC
```

```
TAU10(1)
       1  GGAGATGACA TTTGCTTTGG GCAGAGGCAG CTAGCCAGGA CACATTTCCA
      51  CTATAATTTT ACAAAGTTAA ATTTATAAGC TAGCATTAAG TAAAGTGAAG
     101  TTCCAGCTCC CTTGCTAAAA ATAACTAGAG GTAATAATTG GTATTCAGGT
     151  AACTCATTTA CATCATAATG TGTTGTGAAA A

TAU12/1(2)
       1  TATAAAATAT AAATTATATT ATAAATCATG TATTATTTAT AAAATTATAT
      51  TATAAATTTA TAAAAATATA AATTATATTT TAGGCTTAAT GTATAAGGAA
     101  TATAAATTAT TAATAAGCAT ATGA

TAU 12/1(1)
       1  TGTAATTAAC TGTNCTTGTA GGTCTGTCTT TTATACATGT GTGAGTTTTT
      51  CTTTACAATA GATTCCTAGC ATTGGGATTG CTAGGTCAGA TGGTATGCAC
     101  ATTTGACATT TTGATTGATA GCACCAGATT GCTTTGTTAA AAAATTTTNN
     151  TTTATAGTTT ACATTATCTT TGTACAATAG ATGTTCTCTT TCGAC

TAU 12/2(1)
       1  GGGAAGTGAA TTGAAAATAC TTCTTTNTCA ACATAATTTT NGGGTTTTGA
      51  AATTGTGTTT GGGTTTTCAG GAAATTGGTG GTAATCTTGT ATTAGCTGAA
     101  AAAAAGTGAA TTTTAAAATT CTCAGTGAAG AAGCAAATGA TTTATTTTTC
     151  ATAGA

TAU12/3(2)
       1  TGTTCTGGTA ACTGTTCTAA TTGTGTCTTT GTTACTTCCA GTGCAACCCT
      51  TTCAGGTAAG

TAU12/3(1)
       1  CTAAAGAACT TGGTATCTCT ATTAAAGCAC ACGAACCTCC AAGGAAAATA
      51  GAGCGATTTA CTCTTCTCAT ATCAGTGCAT ATTTATAAGA AGCACGGAGT
     101  CA

TAU13/1(1)
       1  AGTCATCAAT TCCTTTTTAT CTGTAATTAC ACATTTGTTT TTATTTCAAA
      51  GTAATTATAA GGTGTTATAT TGCATATAAT CAGAAAACTA AATGGAAATA
     101  AAATTTTAGT AAGCCCGGCC CCTTTGACCG ATACAGAAAA CTTGA

TAU 13/3(2)
       1  TATATGGCAG TCTAAAGCAT CAAAGATTTG CATCAACATC TTTCATTTTA
      51  GACATCTCCT TGCAATGTAA AATATCATGT ATCAACAACA TCTGGTGCAA
     101  ATCCATGAGT CTAACTCGAC ATTCATCTTA GCTCGATTAT TATTCCTTCG
     151  TACAGTCGAT GTAAACAATA CAGAAAGAGG ATTATTAAGA ACAGTTT
```

TCU9/1(2)

```
   1  ACATAACAGC TTTTATACAA TGATAAGGAC ATATCATTTG TTTACAAAGA
  51  AAGTCTAAAA TTTCAAGAAC ATTCAAAGAG CTAACACAGT AAAGGTCATG
 101  CAAGTTCTAG AATAGTGAAT CATGACAGAA CTCATTCATT TTATCCTTTA
 151  TCTCC
```

TCU9/2(2)

```
   1  AAGTATGGGT AGCTAAATTT GCATTAAATT AAAAGTACAT ATAATGCAAC
  51  ACCACTCTAC ATCTGTATAC CTACGAATGT ATGTGTACTA CACACCCTTA
 101  AAATGTTTTT CAAAGTCTTA ATATATTAGA ACATGTTTTC ATTTTTTCAT
 151  GGGATGTTAA TACTATTCTA TGATTAAGAA AATACTAG
```

TCU10(2)

```
   1  AATACAGTTA TTCTAGCTTT TCATATTCAA TTTGAATGAT CAGAAAAGTA
  51  TATTAGTCAC ACAGAATTAA ATATTTTAGA TAGTAAGAAT C
```

TCU14(1)

```
   1  ATCCTTAGTA AGTGGATTTT GGGGAAAAAA GCACCTGGGC TTCTGGTTCT
  51  TTTTGATAAT ATATAAAATT ATTCATTATG AGGTTGCAGT TGTTTGCAAA
```

TCU14(2)

```
   1  GAAGTGAAAG TCAGCCCTTT AGCTATTATT TATTGCTTTA TTAGAGCAGA
  51  GGGAAGTGAC ACTCATTGCC TTCACAGAGC TCTGCAGAAA TATATGCACA
 101  GAGTGGTCAA TGCCAACATC TGAGTAAGTC TTCCAAA
```

TGO20(2)

```
   1  CAGAACATTA GGATTTATTC CTTGATTAGT TCAAATGATT TCAACAGCTG
  51  AATTCCTTGA GATGTGTAAG GCAGGTTGGT CCTTTGGATG GACTGTAGAC
 101  TGAAACTTCC TATAACTGTA GTGATATGTA CACAGCTACA TAGCAAAGTG
 151  CTTCATTATG AAAATGAAGA A
```

TGO20(1)

```
   1  CAGTGTGAGA GTCTCATTTC TATGCACAGT GTTTCTCAGG AGGATGGAGC
  51  TAGTTAGCTG TCTGTTGTCT GTAGCCCAGC TTGATAATGG AACTATACAG
 101  CGAAGAGACA ATCTCTGGCA AGTTTTTGTA GAA
```

TGU5(C)

```
   1  TTAGAGTAAA ATTCCAAATA AATGCTTTGC TCCAAAATTA CACTAACCAG
  51  GCTGGGTCTC TATCATACAT CTTCAATACC CTCAAACCTA GATTGTAAAG
 101  TGAAAAAAGT GATTAGCNNT TCCATTTGTT CATTCTGTCA CTCACATTCT
 151  TAGGCATTTT AAGGATGAGC AACCTTTGTT TCAGAAAGGG TAAGTAATTA
 201  GCCCCCTGGA GGTTACATAG TTATAATTTA GTCTTCAGAA TCCGTTCGAA
 251  GGGNNNNGTT ACTATTTTTA AGATAATTAG AACCCACCTT GTAGCAATAA
 301  AAGTTTTCTT GTCTTTG
```

TTO20/1(C)

```
   1  CCACCAGCCT ACTGATCAGC TGGGATGCTC CTGCTGTCAC AGTGAGATAT
  51  TACAGGATCA CTTACGGAGA AACAGGAGGA AATAGCCCTG TCCAGGAGTT
 101  CACTGTGCCT GGGAGCAAGT CTACAGCTAC CATCAGCGGC CTTAAACCTG
 151  GAGTTGATTA TACCATCACT GTGTATGCTG TCACTGGCCG TGGAGACAGC
 201  CCCGCAAGCA GCAAGCCAAT TTCCATTAAT TACCGAACAG AAATTGACAA
 251  ACCATCCCAG ATGCAAGTGA CCGATGTTCA AGACAACTGT TTTAATAAAA
 301  GATTTACATT CCAC
```

TTO20/2(2)

```
   1  TTGGTACCAC AGTCACAGAA CTGGGGGTCA TTTTCTAGAT GAAACAAACG
  51  GAACAAGTTC TCTTCCAACA AAGAAATGTA CTGTAGAAAT TAATTTCCTC
 101  CATGAATTTT ATATATTGTG TACAAATATA AGGTATGTAT CTGAATACAA
 151  AG
```

TTU2/1(2)

```
   1  CTAGAACTTC CAAAGGCTGC TTGTCATAGA AGCCATTGCA TCTATAAAGC
  51  AACGGCTCCT GTTAAATGGT ATCTCCTTTC TGAGGCTCCT ACTAAAAGTC
 101  ATTTGTTACC TAAACCTTAT GTGCCTTAAC AGGCCAATGC TTCTCG
```

TTU 2/2(C)

```
   1  AACCAGTATT TCAAAACTAT TATCTGGATT CAAGATTAGT GTGTAAAGAT
  51  TGTTTTCTTA TCAGTAAAAT AGGTCTTCAG ATCTGCATCT GGCCTCTTAG
 101  CATGTTTTTC TTCATAGATA CCCGTTTTGG GGTTTTTGCG TCGGAAGATG
 151  AAGTGCAGTT TATAGTCCTC TCCACATTTA TCTG
```

TTU3(1)

```
   1  GGGTAGAAAG CTGAATAATT TATGAAGGAG AGGGGTCAGG GTTGATTCGG
  51  GAGGACCTAT TGGTGCGGGG GCTTTGTATG ATTATGGGCG TTGATTAGTA
 101  GTAGTTACTG GTTGAACATT GTTTGTTGGT GTATATATTG TAATTGAGAT
 151  TGCTCGGGGG AATAGGTTAT GTGATTAGGA GTAGGGTTAG GATGAGTGGG
 201  AAG
```

TTU 5/1(2)

```
   1  GACAAAAAAA AAAAAACAGG TTTTAAAGCT AGAAATGAAA AGCTACTTAA
  51  GTATCTTAAA GGATAAGTTA CTTTATTATA CACTAGAAAC ATACACAATA
 101  GCTGAAAACT TAAAAAATCT CACACTGCTG AATGTCTCTG CTGGCTG
```

TTU5/2(2)

```
   1  GCATCCATTG TACATTGTTT GGTTTGAGGT TACCATGAGG CCTGTAAATA
  51  CTATCTTATA ATTTATTATT TCAACCTGAT AAAACTTAAC ACTATTTGCA
 101  TAAACAAACA AACGAAAA
```

18. Use of a DNA according to claim 10 or parts thereof or a gene isolated according to claim 13 or 14 or parts thereof for the diagnosis, prophylaxis or therapy of IL-1β mediated diseases of connective tissues, in particular osteoarthritis or rheumatoid arthritis.

19. Use of a gene isolated according to claim 13 to 14 for the production of a pharmaceutical.